

Remarks

I. Status of the Claims

Claims 1-17 are pending and under consideration. Claims 1 and 2 are amended for grammatical clarity. Thus, after entry of this Amendment, claims 1-17 remain pending and under consideration. The amendments of the claims and the various rejections raised in the Office Action are discussed in more detail, below.

II. Rejection under 35 U.S.C. §103

Claims 1-17 were rejected under 35 U.S.C. §103(a) as allegedly obvious over US Patent No. 5,430,054 to Qian *et al.* (hereinafter "Qian") in view of US Patent No. 5,192,817 to Takaishi *et al.* (hereinafter "Takaishi") and in further view of Beroza 1953 and Beroza 1952 (JACS 1953, 75, 44-49 and JACS 1952, 74, 1585-1588). This rejection is respectfully traversed.

A. The Present Claims

The present claims relate to a method for purifying an extract of *Tripterygium wilfordii* plant material containing triptolide and related compounds. The extract is formed by (a) extracting plant material with aqueous ethanol, and concentrating to obtain a residue; and (b) forming a slurry of this residue in a chlorinated organic solvent; partitioning the slurry with water for a period of about 10 mins-10 hours; and then removing the water; and the purifying comprises the steps of: (c) further partitioning the slurry with an aqueous solution of base, removing the aqueous solution of base, and removing at least a portion of the organic solvent from the slurry; (d) washing the product of step (c) with a lipophilic solvent; and (e) eluting the washed product from step (d) from a silica gel adsorbent.

B. The Cited Art

QIAN is directed to methods of preparing a male antifertility agent composed of a diterpene lactone compound obtained from plants of the genus *Tripterygium*, and the use of the compounds as an antifertility agent.

TAKAISHI describes phenanthrene derivatives and a method of extracting *Tripterygium Wilfordii* Hook fil. var. Regelii Makino with an ordinary polar solvent such as methanol, ethanol or the like, concentrating the resulting extract under reduced pressure to obtain a primary extract, and collecting an intended compound from the primary extract using various methods utilizing the physical and chemical properties of the intended compound.

BEROZA 1953 AND 1952 describe a process for removing alkaloids from the initial extracts of *Tripterygium Wilfordii*.

C. Analysis

As acknowledged by the Examiner (page 4 of Office Action), the disclosure in the primary reference, Qian, differs from the present claims by Applicants' additional step of further partitioning the extracted residue / slurry with an aqueous solution of base, an additional step implemented to remove significant amounts of impurities at an early stage of the process, thereby increasing yields and reducing production costs. However, the Examiner contends that "(i)t would be obvious to an artisan of ordinary skill in the art to incorporate a saponification step in the purification of *Tripterygium wilfordii* plant material in order to remove undesired wilforine, wilfordine, wilforgine and wilfortrine and derivatives thereof in view of the references of Beroza" (page 5 of Office Action). Applicants respectfully disagree.

Applicants urge the Examiner to reconsider Applicants' claim 1. From the Examiner's statement, it appears that the rejection is based on hindsight recognition and the disclosure of the Beroza reference rather than Applicants' actual claim. Applicants' actual claim 1 is directed to a method for purifying an extract of *Tripterygium wilfordii* (Tw) plant material containing triptolide and related compounds, and does not recite "to remove undesired wilforine, wilfordine, wilforgine and wilfortrine and derivatives."

Applicants' claimed subject matter produces unpredictable results

The Office examination guidelines following the Court decision in *KSR International Co. v. Teleflex Inc.*, 82 USPQ2d 1385, 1385 (US 2007) indicate that an issue to consider in assessing obviousness is whether a combination of prior art elements yields "predictable results." See *Federal Register*, Vol. 72, No. 195, October 10, 2007. Further, as noted in M.P.E.P. § 2143.02, in an obviousness determination, the predictability of a technology is determined at the time the invention was made. "Whether an art is predictable or whether the proposed modification or combination of the prior art has a reasonable expectation of success is determined at the time the invention was made. *Ex parte Erlich*, 3 USPQ2d 1011 (Bd. Pat. App. & Inter. 1986).

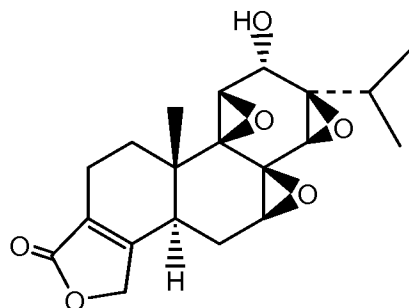
As noted in the specification (see PCT Publication WO 2005/077008 at page 2, lines 5-6), the state of the art in the field of isolating native compounds from plant material typically required laborious extraction and purification procedures. Prior to Applicants' disclosed method, known methods typically produced "an extract which retains large quantities of undesired materials, which then must be removed chromatographically, requiring large investments of time and materials. In view of the therapeutic utility of these compounds, higher efficiency methods for isolation and purification are desired." Page 2, lines 21-25. The teachings of the cited references do not avoid these additional laborious purification steps.

In contrast, Applicants' approach simplified known purification procedures to relatively few process steps (in comparison to conventional natural products isolations), using a base treatment step to remove undesired impurities. And while it is true that acid/base washing was known in the literature, it was also known that such a procedure typically opens epoxide rings (see **Exhibits A and B**, by March, and Carey & Sundberg, attached). The art and knowledge of the skilled artisan actually taught away from the use of base. In the March reference from 1992, it states "Epoxide ring opening reactions are catalyzed by acids or bases and is a convenient method for the preparation of vic-diols." (March *Advanced Organic Chemistry*, 4th Ed.; Wiley, 1992, p 376 - 377). Furthermore, in a journal article (attached as **Exhibit C**), by Chen *et al.* *Journal of Controlled Release* 98 (2004) 427–436, page 432 states:

A long-term storage of triptolide in an aqueous environment may result in a potential tendency of hydrolysis, because triptolide has unstable ether-bond. Therefore, it is disadvantageous that triptolide was formulated to an aqueous environment. The rate of hydrolysis of triptolide is also influenced significantly by pH value. Especially when pH value is less than 3 or more than 8, the hydrolysis of triptolide can be promoted.

Again, it was known that under basic conditions, the 12, 13 epoxide of triptolide is subject to base induced nucleophilic attack, in this case by (–OH), yielding 12, 13 dihydroxy triptolide and related molecules which are inactive. For example, even after Applicants' effective filing date, base treatment of triptolide was understood by skilled artisans to result in a loss of bioactivity:

Triptolide **1** is a stable compound, but in basic conditions (pH 7.4, such as treatment with HNMe₂) it will isomerize to isotriptolide **68**, with loss of bioactivity.



Isotriptolide

(See Xu, R.S., Fidler, J.M., Musser, J.H., Bioactive compounds from *Tripterygium wilfordii*, pp. 773-802, in *Studies in Natural Products Chemistry*, Volume 32, Rahman, Atta-ur, Ed., Elsevier, Nov (2005)).

Nonetheless, in spite of these teachings away from the use of base, Applicants tried and found that a base partitioning step removes impurities without destroying the desired triptolide and related compounds. Applicants' specification clearly teaches, at an early step in the

purification process, this base partitioning step as well as the use of several bases and counterions:

In the aqueous solution of base, the base is preferably a water soluble hydroxide, carbonate or bicarbonate having a counterion selected from lithium, sodium, potassium, cesium, ammonium, and tetraalkylammonium, where alkyl is preferably C₁-C₄ alkyl. Suitable bases include, for example, NaOH, KOH, NaHCO₃, KHCO₃, Na₂CO₃ and K₂CO₃. The solution may be selected, accordingly, from 0.1N - 2.5N aqueous NaOH, 0.1N - 2.5N aqueous KOH, 10% - 15% aqueous NaHCO₃, and 12% - 18% aqueous KHCO₃. The base partitioning is generally carried out for about 2 days, following a brief (e.g. 5-20 minutes, typically about 10 minutes) period of stirring. Optionally, following the removal of the aqueous solution of base, and prior to the removal of all or a portion of the organic solvent, the organic solvent is washed with a dilute aqueous acidic solution.

(See instant application at page 4, lines 11-21).

It is also known that with simple inorganic bases, the stronger the base as measured by pH, the better the nucleophile. The pHs of concentrated inorganic bases KOH, NaOH, Na₂CO₃, NaHCO₃ are 14, 14, 10 and 7.5, respectively. Thus, one skilled in the art would predict that the strong bases KOH and NaOH would cause significant inactivation of triptolide, whereas the weaker bases NaHCO₃ and Na₂CO₃ would be less prone to inactivate the desired triptolide and related compounds. One skilled in the art would not expect that the use of a base wash step in the preparation of an extract of Tw plant material could remove impurities without destroying the epoxides of the desired triptolide and related compounds.

Base treatment does indeed open the 12, 13 epoxide ring and the resulting product 12, 13, 14-trihydroxytriptolide is inactive. However, as shown in the post-filing data presented hereinbelow, counter to the expectation that the stronger nucleophiles would inactivate triptolide, Applicants surprisingly found that the stronger bases were most effective at increasing the yield of triptolide and related compounds.

Inorganic base	Concentration	PH	Triptolide (% wt/wt)
No base	-	~7	0.3015
NaOH	1.0N	14	0.7398
NaOH	0.3 N	14	0.8589
KOH	1.0N	14	0.7042
KOH	0.3N	14	0.7563
NaHCO ₃	10%	9	0.5692
NaHCO ₃	5%	8	0.4498
Na ₂ CO ₃	10%	10	0.4671
Na ₂ CO ₃	5%	10	0.4034

(mixture treated has no or little buffering capacity). Of the inorganic bases tried, the range from best to worst (in terms of the amount of triptolide obtained) is NaOH, KOH, NaHCO₃, and Na₂CO₃.

Thus, in spite of this teaching away and the general knowledge in the art and expectation of the skilled artisan that an acid/base wash would open the epoxide rings, Applicants tried and found that a base partitioning step could be used to achieve increased efficiency in the preparation of an extract of *Tripterygium wilfordii* (Tw) plant material containing triptolide and related compounds. In Applicants' method, the extract, or more properly, the slurry obtained from the extract, obtained as set forth in steps (a) and (b), is partitioned by treatment with aqueous base. The partitioning step is followed by removal of aqueous base, removal of at least a portion of the remaining chlorinated organic solvent, a lipophilic solvent wash, and elution of the isolated material from silica gel. Applicants' method achieves the unexpected effect of increasing efficiency in the preparation of an extract of *Tripterygium wilfordii* (Tw) plant material containing triptolide and related compounds, such as triptolide and/or 16-hydroxytriptolide, triptolide, triptochlorolide and triptonide, and isolation of these compounds. Applicants found that treatment of the extract with aqueous base does not reduce the extractable amount of triptolide recovered from Tw tissues. Rather, recovery of triptolide is increased over separation methods absent a base-treatment step. This feature of the method is utterly unexpected.

Additionally, Applicants' specification clearly teaches several process advantages, at page 5, lines 4-14 of the specification, which states:

the method includes treatment of an initial organic extract with aqueous base, which removes a significant amount of impurities at an early stage of the process, thereby increasing yield and reducing production costs. The base treatment effectively removes acidic or weakly acidic compounds (e. g. celastrol, triptochlorolide, triptochlorolide G, 3-hydroxyoleanolic acid, polypunonic acid, tripterygic acid A, and phenolic compounds such as triptonoterpene, hypolide, triptophenolide, and triptonodial) from the organic TW extract. The base treatment also remove "oily" impurities from the extract via saponification and/or hydrolysis. This step has been found to remove about 70% of the undesired impurities from the extract, including oily materials whose removal typically generates a large majority of the cost of subsequent purification using prior art methods. (emphasis added).

Thus, in addition to removing undesired impurities at an early stage of the process, the treatment of the initial organic extract with aqueous base has several further advantages. One advantage of Applicants' simplification over conventional methods is noted at page 5, lines 15-19 of the specification:

The method of the invention also includes an extraction with a lipophilic solvent, such as cyclohexane or a similar hydrocarbon-based solvent, e.g. hexanes, pentanes, petroleum ether, etc., to remove less polar impurities from the extract. This step further simplifies the subsequent chromatographic purification steps, by removing components which would otherwise typically be removed chromatographically.

Overall, and in summary, Applicants' method produced unexpected results, and surprisingly left the triptolide epoxide ring intact without adversely impacting product yields. The efficacy of a base wash was unexpected, since one skilled in the art would expect base treatment to hydrolyze the epoxide ring of the desired products, making recovery of triptolide and its related compounds in intact form impossible. Treatment with base was not only surprising with respect to the lack of base-catalyzed epoxide ring opening, but also solved several other processing problems -- increasing product yield, reducing the number of purification steps required, circumventing having to deal with removal of oily impurities, and reducing processing costs. Unexpectedly, recovery of triptolide and related compounds was found to be enhanced by Applicants' method over traditional separations approaches. Applicants found that treatment of the extract with aqueous base does not reduce the extractable amount of triptolide from Tw tissues. Because the percentage of crude extract containing the desired triptolide and related compounds is increased and isolation steps of the process are eliminated, production costs are reduced and the final yield of triptolide and related compounds is increased.

Because Applicants have provided evidence of nonobviousness of the claimed subject matter, and because the claimed method produces more than could be predicted from the cited references, the presently claimed subject patentably defines over the cited references, and respectfully request withdrawal of the rejection under 35 U.S.C. § 103.

CONCLUSION

In view of the foregoing, claims 1-17 are believed to satisfy all of the criteria for patentability and are in condition for Allowance. An early indication of the same is therefore kindly requested.

No fees beyond the fee for a three-month extension of time are believed to be due in connection with this Amendment. However, the Commissioner is authorized to charge any additional fees that may be required, or credit any overpayment, to King & Spalding LLP Deposit Account No. 50-4616.

If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is encouraged to call the undersigned at (650) 590-1932.

Respectfully submitted,
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